REMARKS/ARGUMENTS

Claims 28-35 and 38-40 are pending in this application.

I.   Claim Rejections Under 35 U.S.C. §101 and 35 U.S.C. §112, First Pargraph

Claims 28-35 and 38-40 remain rejected under 35 U.S.C. §101 allegedly "because the claimed invention is not supported by either a credible, specific and substantial asserted utility or a well established utility." (Page 2 of the Office Action mailed October 6, 2005).

Claims 28-35 and 38-40 also remain rejected under 35 U.S.C. §112, first paragraph, allegedly "since the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility for the reasons set forth above, one skilled in the art clearly would not know how to use the claimed invention." (Page 19 of the Office Action mailed October 6, 2005).

Applicants submit, as discussed below, that not only has the PTO not established a *prima facie* case for lack of utility, but that the polypeptides of Claims 28-35 and 38-40 possess a specific and substantial asserted utility, and that based upon this utility, one of skill in the art would know how to use the claimed polypeptides without any further experimentation.

The gene amplification data disclosed in Example 143 establishes a credible, substantial and specific patentable utility for the PRO1759 polypeptides

First of all, Applicants respectfully maintain the position that the specification discloses at least one credible, substantial and specific asserted utility for the claimed PRO1759 polypeptides for the reasons previously set forth in Applicants' Responses filed on February 2, 2005, July 21, 2005 and November 30, 2005.

Furthermore, as first discussed in Applicants' Response of February 2, 2005, Applicants respectfully submit that Applicants rely on the gene amplification data for patentable utility of the claimed PRO1759 polypeptides, and that the gene amplification data for the gene encoding the PRO1759 polypeptide is clearly disclosed in the instant specification under Example 143.

It was well known in the art at the time the invention was made that gene amplification is an essential mechanism for oncogene activation. The gene amplification assay is well-described in Example 143 of the present application. Example 143 discloses that the inventors isolated genomic DNA from a variety of primary cancers and cancer cell lines that are listed in Table 8,

-6-

including primary lung and colon tumors of the type and stage indicated in Table 7. As a negative control, DNA was isolated from the cells of ten normal healthy individuals, which was pooled and used as a control. Gene amplification was monitored using real-time quantitative TaqMan™ PCR. Table 8 shows the resulting gene amplification data. Further, Example 143 explains that the results of TaqMan™ PCR are reported in ΔCt units, wherein one unit corresponds to one PCR cycle or approximately a 2-fold amplification relative to control, two units correspond to 4-fold amplification, 3 units to 8-fold amplification etc.

A ΔCt value of at least 1.0 was observed for PRO1759 in at least three of the tumors listed in Table 8. PRO1759 showed approximately 1.11-1.51 ΔCt units which corresponds to $2^{1.11}$-$2^{1.51}$ fold amplification or 2.16 fold to 2.85-fold amplification in lung tumors HF000842 and HF001296, and in colon tumor center HF000795. (See Table 8 of the specification). Accordingly, the present specification clearly discloses overwhelming evidence that the gene encoding the PRO1759 polypeptide is significantly amplified in lung and colon tumors.

A *prima facie* case of lack of utility has not been established

The Examiner asserts that "the specification provides data showing a very small increase in DNA copy number in two different types of tumor tissue (lung and colon)....However, there is no evidence regarding whether or not PRO1759 mRNA or polypeptide levels are also increased in these cancers." The Examiner further asserts that "what is often seen is a lack of correlation between DNA amplification and increased peptide levels." (Page 4 of the Office Action mailed October 6, 2005).

In support of these assertions, the Examiner refers to previously cited papers by Pennica *et al.*, Haynes *et al.*, Chen *et al.* and Hu *et al.* The Examiner also cites additional papers by Madoz-Gurpide *et al.*, Celis *et al.*, Steiner *et al.* and Feroze-Merzoug *et al.* in support of the assertion that "the state of the art is such that polypeptide levels cannot be accurately predicted from mRNA levels." (Page 5 of the Office Action mailed October 6, 2005).

Applicants respectfully remind the Examiner that the evidentiary standard to be used throughout *ex parte* examination of a patent application is a preponderance of the totality of the evidence under consideration. Accordingly, Applicants submit that in order to overcome the presumption of truth that an assertion of utility by the applicant enjoys, the Examiner must establish that **it is more likely than not** that one of ordinary skill in the art would doubt the truth

-7-

of the statement of utility. Therefore, it is not legally required that there be a "necessary" correlation between the data presented and the claimed subject matter, such that the amplification of every possible gene inevitably results in protein over-expression. The law requires only that one skilled in the art should accept that such a correlation is **more likely than not to exist**. Applicants respectfully submit that when the proper evidentiary standard is applied, a correlation must be acknowledged.

Applicants respectfully submit that, for the reasons previously set forth in the Applicants' response filed on February 2, 2005, the teachings of Pennica *et al.* are specific to *WISP* genes, and say nothing about the correlation of gene amplification and protein expression in general.

The Examiner reiterates that Haynes *et al.* state that "[p]rotein expression levels are not predictable from the RNA expression levels." (Page 7 of the Office Action mailed October 6, 2005). Applicants respectfully point out that, on the contrary, Haynes *et al.* never indicate that the correlation between mRNA and protein levels does not exist. Haynes *et al.* only state that "protein levels cannot be *accurately* predicted from the level of the corresponding mRNA transcript" (See page 1863, under Section 2.1, last line, emphasis added). This result is expected, since there are many factors that determine translation efficiency for a given transcript, or the half-life of the encoded protein. Not surprisingly, Haynes *et al.* concluded that protein levels cannot always be accurately predicted from the level of the corresponding mRNA transcript in a single cellular stage or type when looking at the level of transcripts across different genes.

Importantly, Haynes *et al.* did not say that for a single gene, a change in the level of mRNA transcript is not positively correlated with a change in the level of protein expression. Applicants have asserted that increasing the level of mRNA for a particular gene leads to a corresponding increase for the encoded protein. Haynes *et al.* did not study this issue and says absolutely nothing about it. One cannot look at the level of mRNA across several different genes to investigate whether a change in the level of mRNA for a particular gene leads to a change in the level of protein for that gene. Therefore, Haynes *et al.* is not inconsistent with or contradictory to the utility of the instant claims, and offers no support for the PTO's rejection of Applicants' asserted utility.

-8-

Furthermore, Applicants note that contrary to the Examiner's statement, Haynes teaches that *"there was a general trend* but no strong correlation between protein [expression] and transcript levels" (See page 1863, under Section 2.1, emphasis added). For example, in Figure 1, there is a positive correlation between mRNA and protein amongst *most* of the 80 yeast proteins studied but the correlation is not linear, hence the authors suggest that one cannot *accurately* predict protein levels from mRNA levels. In fact, very <u>few</u> data points deviated or scattered away from the expected normal or showed a lack of correlation between mRNA: protein levels. Thus, the Haynes data meets the "more likely than not standard" and shows that a positive correlation exists between mRNA and protein. Therefore, Applicants submit that the Examiner's rejection is based on a misrepresentation of the scientific data presented in Haynes *et al.*

Haynes *et al.* may teach that protein levels cannot be "accurately predicted" from mRNA levels in the sense that the <u>exact numerical amounts</u> of protein present in a tissue cannot be · determined based upon mRNA levels. Applicants respectfully submit that the PTO's emphasis on the need to "accurately predict" protein levels based on mRNA levels misses the point. The asserted utility for the claimed polypeptides is in the diagnosis of cancer. What is relevant to use as a cancer diagnostic is <u>relative</u> levels of gene or protein expression, not absolute values, that is, that the gene or protein is differentially expressed in tumors as compared to normal tissues. Applicants need only show that there is a correlation between mRNA and protein levels, such that mRNA overexpression generally predict protein overexpression. <u>A showing that mRNA levels can be used to "accurately predict" the precise levels of protein expression is not required</u>.

The Examiner further cites Feroze-Merzoug *et al.*, to the effect that a lack of correlation between mRNA and protein is evident even in yeast. These conclusions are based upon a citation to a paper by Gygi *et al.* (Mol. Cell. Biol. 19:1720-1730 (1999); copy enclosed as Exhibit 1). Gygi *et al.*, however, clearly teach that "there was a <u>general trend</u> of increased protein levels resulting from increased mRNA levels." (Emphasis added. See page 1726, left column, second paragraph and Figure 5). Gygi *et al.* further states that the "correlation coefficient for the whole data set (106 genes) was 0.935." In addition, Gygi *et al.* state that the correlation coefficient for genes where the message level was below 10 copies/cell was 0.356 and for most abundant proteins was 0.94. (See page 1726, second paragraph and page 1727, left column, second paragraph). Therefore, Gygi *et al.* disclose positive correlations for both lower abundance and

-9-

higher abundance proteins. Gygi *et al.* clearly teach that there is a general trend of increased protein levels from increased mRNA levels for all levels of mRNA expression. Thus, again, Feroze-Merzoug *et al.* appear to be focusing on "accurately predicting" the precise levels of protein expression, which is not required for utility as a cancer diagnostic.

Applicants further respectfully submit that Futcher *et al.* (Mol. Cell. Biol. 19:7357-7368 (1999); copy enclosed as Exhibit 13) also analyzed the yeast proteome using 2D gel electrophoresis, gathering quantitative data on protein abundance for about 1,400 spots. This data was compared to mRNA abundance for each gene as determined both by SAGE (serial analysis of gene expression) and by hybridization of cRNA to oligonucleotide arrays. The authors concluded that "**several statistical methods show a strong and significant correlation between mRNA abundance and protein abundance**" (page 7360, col. 2; emphasis added).

The authors note that Gygi *et al.* completed a similar study that generated broadly similar data, but reached different conclusions. Futcher *et al.* point out that "the different conclusions are also partly due to different methods of statistical analysis, and to real differences in data." Futcher *et al.* note that Gygi *et al.* used the Pearson product-moment correlation coefficient (r_p) and point out that "a calculation of r_p is inappropriate" because the mRNA and protein abundances are not normally distributed (page 7367, col. 1). In contrast, Futcher *et al.* used two different statistical approaches to determining the correlation between mRNA and protein abundances. First, they used the Spearman rank correlation coefficient (r_s), an nonparametric statistic that does not require the data to be normally distributed. Using the r_s, the authors found that mRNA abundance was well correlated with protein abundance ($r_s = 0.74$). Applying this statistical approach to the data of Gygi *et al.* **also** resulted in a good correlation ($r_s = 0.59$), although the correlation was not quite as strong as for the Futcher *et al.* data. In a second approach, Futcher *et al.* transformed the mRNA and protein data to forms where they were normally distributed, in order to allow calculation of an r_p. Two types of transformation (Box-Cox and logarithmic) were used, and **both** resulted in good correlations between mRNA and protein abundance for Futcher *et al.*'s data.

Futcher *et al.* also note that the two studies used different methods of measuring protein abundance. Gygi *et al.* cut spots out of each gel and measured the radiation in each spot by scintillation counting, whereas Futcher *et al.* used phosphorimaging of intact gels coupled to

-10-

image analysis. Futcher *et al.* point out that <u>Gygi *et al.* may have systematically overestimated the amount of the lowest-abundance proteins</u>, because of the difficulty in accurately cutting out very small spots from the gel, and because of difficulties in background subtraction for small, weak spots.

In addition, Futcher *et al.* note that they used both SAGE data and RNA hybridization data to determine mRNA abundances, which is most helpful to accurately measure the least abundant mRNAs. As a result, while the Futcher data set "maintains a good correlation between mRNA and protein abundance even at low protein abundance" (page 7367, col. 2), the Gygi data <u>shows a strong correlation for the most abundant proteins</u>, but a poor correlation for the least abundant proteins in their data set. Futcher *et al.* conclude that **"the poor correlation of protein to mRNA for the nonabundant proteins of Gygi *et al.* may reflect difficulty in accurately measuring these nonabundant proteins and mRNAs, rather than indicating a truly poor correlation *in vivo*"** (page 7367, col. 2; emphasis added). These lowest abundance proteins are precisely those pointed out by the Examiner as being shown in the inset of Figure 1. Thus while these lowest abundant proteins do show a poor correlation, this is almost certainly due to the less accurate methods used to measure the abundance of these proteins, and **not** to any actual lack of correlation.

Applicants further note that Feroze-Merzoug *et al.* looked specifically at androgen regulated genes, which were not necessarily associated with cancer. The expression of these genes clearly involves different biological process than in lung or colon tumor development. Therefore, even if the teaching of Feroze-Merzoug *et al.* accurately reflects the correlation between mRNA and protein for the particular system studied, it does not apply to the lung and colon cancer diagnostic assays of the present application.

The reference by Chen *et al.*, as discussed in Applicants' Response filed July 21, 2005 is not applicable to the present application, in part because the Chen paper does not account for different expression in different tissues or different stages of cancer. Further, a review of the actual data presented in Tables I and II of the Chen paper demonstrates that it is more likely than not that protein levels <u>will correlate</u> with mRNA expression levels. Once again, the Examiner focuses on statements in the Chen paper to the effect that protein levels cannot be "predicted" from mRNA levels, and that mRNA expression patterns are "insufficient for understanding the

-11-

expression of protein products." (Page 8 of the instant Office Action). As discussed above, there is no requirement that protein levels be "accurately predicted."

The same authors in Chen *et al.*, published a later paper, Beer *et al.*, which described gene expression of genes in adenocarcinomas and compared that to protein expression. The results demonstrate that the authors of the Chen paper agree that microarrays provide a reliable measure of the expression levels of the gene and can be used to identify genes whose overexpression is associated with tumors. The Examiner asserts that Beer *et al.* is not relevant to the instant application because the authors also did additional northern blot and immunohistochemistry experiments for three of their identified genes. The Examine asserts that "the specification of the instant application does not complement the low (2-fold) PRO1759 gene expression data with any mRNA or protein studies." (Pages 9-10 of the Office Action mailed October 6, 2005). In support of the role of proteomics as a "complementary technology" to DNA microarrays, the Examiner cites references by Madoz-Gurpide *et al.*, Celis *et al.* and Steiner *et al.*, in addition to Feroze-Merzoug *et al.*

Applicants respectfully point out that Madoz Gurpide *et al.* state only that is it "unclear" how well RNA levels reported correlate with protein levels. In support of this assertion, the authors cite only a single reference, the same paper by Chen *et al.* previously cited by the Examiner, which Applicants have already discussed. Madoz Gurpide *et al.* also acknowledge that DNA microarray studies, such as those carried out by Beer *et al.*, specifically cited by the authors at page 52, "**justify the use of this technology for uncovering patterns of gene expression that are clinically informative**" (page 53; emphasis added).

Applicants respectfully submit that while proteomics is indeed a complementary technology to DNA microarrays, this does not mean that proteomic experiments are <u>required</u> in addition to measurements of mRNA levels to determine protein expression. The cited references make clear that proteomic techniques are useful to obtain information <u>beyond</u> expression levels, such as the protein's activation state, posttranslational modifications, and subcellular localization. For example, Madoz-Gurpide *et al.* explain that mRNA expression alone does not provide information regarding "activation state, post-translational modification or localization of corresponding proteins" (page 168, col. 1). Celis *et al.* note that "proteomics addresses problems that cannot be approached by DNA analysis, namely, relative abundance of the protein product,

-12-

post-translational modification, subcellular localization, turnover, interaction with other proteins as well as functional aspects" (page 6, col. 2).

While this additional information may be useful in elucidating the detailed biological function of a protein, it is not required to establish utility of a protein as a marker for cancer. The claimed PRO1759 polypeptides can be used in cancer diagnosis without any knowledge regarding the function or cellular role of the polypeptides. Applicants submit that the law clearly states that "it is not a requirement of patentability that an inventor correctly set forth, or even know, how or why the invention works." *Newman v. Quigg*, 11 U.S.P.Q.2d 1340 (Fed. Cir. 1989). Accordingly, the disclosure or identification of the mechanism by which PRO1759 is associated with cancer is not required in order to establish the patentable utility of the claimed PRO1759 polypeptides. Thus while Madoz-Gurpide *et al.* note that it is "more difficult to develop an understanding of disease at a mechanistic level using DNA microarrays," (page 53) this is not relevant to Applicants' assertions of utility, since, as discussed above, it is not necessary to understand how or why an invention works in order to demonstrate utility.

Applicants note that the cited references make clear that proteomic data is useful primarily in the areas of drug development, in order to better understand the functional mechanisms of the protein target. These considerations are not relevant to use as a cancer diagnostic. Thus Steiner *et al.* does not even discuss cancer or diagnostics, but is limited to the role of proteomics in drug development and toxicology testing. Celis *et al.* explain that proteomics is useful in combination with arrays "for the entire process of drug development and evaluation." (page 6; col. 1).

Applicants further submit that significant correlations between gene and protein expression are most likely to be observed for genes associated with cancer, since as Celis *et al.* note, "transformation resulted in the abnormal expression of normal genes, rather than in the expression of new ones" (page 11, col. 1). Accordingly, alterations in gene amplification or expression are more likely to be associated with altered protein expression in the case of cancer than in other cases where DNA microarrays are used (for example, the study of androgen-regulated genes described in Feroze-Merzoug *et al.*), because, as explained by Celis *et al.*, the alterations in expression levels of certain normal proteins are part of the process that leads to cancer.

-13-

In their discussion of DNA microarrays and proteomics applied to the same samples, Celis *et al.* cite Orntoft *et al.*, and note that "**in most cases there was a good correlation between transcript and protein levels**" (page 13, col. 1; emphasis added). Celis *et.al.* further explain that those few cases which showed apparent discrepancies may have been due to other causes, such as post-transcriptional processing or degradation of the protein, or the choice of methods used to assess protein expression levels. Celis *et al.* also note that the observation that there is often more change in mRNAs as compared to the proteins may be due to the fact that current technologies detect mainly high abundance proteins, while most of the changes affecting protein levels may involve low abundance proteins. Thus the correlation between mRNA and protein levels may be <u>even higher</u> than typically observed, given these factors.

Finally, the Examiner again cites Hu *et al.* in support of the assertion that mRNA expression is not correlated with protein expression. Applicants respectfully submit that Hu *et al.* does not conclusively show that it is more likely than not that <u>gene amplification</u> does not result in increased expression at the mRNA and polypeptide levels. Applicants respectfully submit that Hu *et al.* manipulated various aspects of the input data in order to minimize the false positives and negatives in their analysis Applicants further submit that the statistical analysis by Hu *et al.* is not a reliable standard because the frequency of citation only reflects the current research interest in a molecule but not the true biological function of the molecule. Finally, the conclusion in Hu *et al.* only applies to a specific type of breast tumor (estrogen receptor (ER)-positive breast tumor) and can not be generalized as a principle governing microarray study of breast cancer in general, let alone the various other types of cancer genes <u>in general</u>. In fact, even Hu *et al.* admit that "[i]t is likely that this threshold will change depending on the disease as well as the experiment. Interestingly, the observed correlation was only found among ER-positive (breast) tumors not ER-negative tumors." (See page 412, left column). Therefore, based on these findings, the authors add, "This may reflect a <u>bias in the literature</u> to study the more prevalent type of tumor in the population. Furthermore, this emphasizes that caution must be taken when interpreting experiments that may contain subpopulations that behave very differently." (*Id.*; emphasis added).

Furthermore, Hu *et al.* <u>did not look for</u> a correlation between changes in mRNA and changes in protein levels, and therefore their results are not contrary to Applicants' assertion that

-14-

there is a correlation between the two. <u>Applicants are not relying on any "biological role" that the PRO1759 polypeptide has in cancer for its asserted utility</u>. Instead, Applicants are relying on the overexpression of PRO1759 in certain tumors compared to their normal tissue counterparts. Nowhere in Hu does it say that a lack of correlation in their study means that genes with a less than five-fold change in level of expression in cancer cannot serve as a <u>diagnostic marker</u> of cancer.

The Examiner asserts that "Applicant is holding Hu *et al.* to a higher standard than their own specification, which does not provide proper statistical analysis such as reproducibility, standard error rates, etc." (Page 11 of the Office Action mailed October 6, 2005). Applicants note that they do not argue that Hu *et al.* lacks reproducibility, standard error rates, etc. for their data, given that Hu *et al.* did a literature survey and conducted no actual experiments of their own. Rather, Applicants' point is that, given the various biases in selecting the data to be considered, as acknowledged by the authors themselves, the collection of data surveyed by Hu *et al.* simply does not demonstrate the conclusion the PTO attempts to reach concerning a <u>general</u> lack of correlation between microarray data and biological significance. Accordingly, Applicants respectfully submit that the Examiner has <u>not</u> shown a lack of correlation between microarray data and the biological significance of cancer genes.

The Patent Office has failed to meet its initial burden of proof that Applicant's claims of utility are not substantial or credible. The arguments presented by the Examiner in combination with the Pennica, Haynes, Hu, and Chen papers, as well as the Madoz-Gurpide, Steiner, Celis and Feroze-Merzoug papers, do not provide sufficient reasons to doubt the statements by Applicants that PRO1759 has utility. As discussed above, the law does not require the existence of a "necessary" correlation between mRNA and protein levels. Nor does the law require that protein levels be "accurately predicted." According to the authors themselves, the data in the above cited references confirm that there is a general trend between protein expression and transcript levels, which meets the "more likely than not standard" and show that a positive correlation exists between mRNA and protein. Therefore, Applicants submit that the Examiner's reasoning is based on a misrepresentation of the scientific data presented in the above cited reference and application of an improper, heightened legal standard. In fact, contrary to what the

-15-

Examiner contends, the art indicates that, if a gene is amplified in cancer, it is more likely than not that the encoded protein will be expressed at an elevated level.

It is "more likely than not" for amplified genes to have increased mRNA and protein levels

Applicants have submitted ample evidence to show that, in general, if a gene is amplified in cancer, it is more likely than not that the encoded protein will be expressed at an elevated level. First, the articles by Orntoft *et al.*, Hyman *et al.*, and Pollack *et al.*, (made of record in Applicants' Response filed February 2, 2005) collectively teach that in general, gene amplification increases mRNA expression. Second, the Declaration of Dr. Paul Polakis, principal investigator of the Tumor Antigen Project of Genentech, Inc., the assignee of the present application, shows that, in general, there is a correlation between mRNA levels and polypeptide levels.

With respect to the correlation between mRNA expression and protein expression levels, Applicants emphasize that the opinions expressed in the Polakis Declaration are all based on factual findings. Thus, Dr. Polakis explains that in the course of their research using microarray analysis, he and his co-workers identified approximately 200 gene transcripts that are present in human tumor cells at significantly higher levels than in corresponding normal human cells. Subsequently, antibodies binding to about 30 of these tumor antigens were prepared, and mRNA and protein levels were compared. In approximately 80% of the cases, the researchers found that increases in the level of a particular mRNA correlated with changes in the level of protein expressed from that mRNA when human tumor cells are compared with their corresponding normal cells. Dr. Polakis' statement that "an increased level of mRNA in a tumor cell relative to a normal cell typically correlates to a similar increase in abundance of the encoded protein in the tumor cell relative to the normal cell" is based on factual, experimental findings, clearly set forth in the Declaration. Accordingly, the Declaration is not merely conclusive, and the fact-based conclusions of Dr. Polakis would be considered reasonable and accurate by one skilled in the art.

Furthermore, without acquiescing to the propriety of this rejection, and merely to expedite prosecution in this case, **Applicants present a second Declaration by Dr. Polakis (Polakis II) that presents evidentiary data in Exhibit B**. Exhibit B of the Declaration identifies 28 gene transcripts out of 31 gene transcripts (*i.e.*, greater than 90%) that showed good correlation

-16-

between tumor mRNA and tumor protein levels. As Dr. Polakis' Declaration (Polakis II) says "[a]s such, in the cases where we have been able to quantitatively measure both (i) mRNA and (ii) protein levels in both (i) tumor tissue and (ii) normal tissue, we have observed that in the vast majority of cases, there is a very strong correlation between increases in mRNA expression and increases in the level of protein encoded by that mRNA." Accordingly, Dr. Polakis has provided the facts to enable the Examiner to draw independent conclusions.

The case law has clearly established that in considering affidavit evidence, the Examiner must consider all of the evidence of record anew.[1] "After evidence or argument is submitted by the applicant in response, patentability is determined on the totality of the record, by a preponderance of the evidence with due consideration to persuasiveness of argument."[2] Furthermore, the Federal Court of Appeals held in *In re Alton*, "We are aware of no reason why opinion evidence relating to a fact issue should not be considered by an Examiner."[3] Applicants also respectfully draw the Examiner's attention to the Utility Examination Guidelines[4] which state, "Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered." The statement in question from an expert in the field (the Polakis Declaration) states: "it is my considered scientific opinion that for human genes, an increased level of mRNA in a tumor cell relative to a normal cell typically correlates to a similar increase in abundance of the encoded protein in the tumor cell relative to the normal cell." Therefore, barring evidence to the contrary regarding the above statement in the Polakis declaration, this rejection is improper under both the case law and the Utility guidelines.

Both Polakis Declarations (Polakis I and II) are further supported by the teachings in Molecular Biology of the Cell, a leading textbook in the field (Bruce Alberts, *et al.*, Molecular

---

[1] *In re Rinehart*, 531 F.2d 1084, 189 U.S.P.Q. 143 (C.C.P.A. 1976); *In re Piasecki*, 745 F2d. 1015, 226 U.S.P.Q. 881 (Fed. Cir. 1985).

[2] *In re Alton*, 37 U.S.P.Q.2d 1578, 1584 (Fed. Cir 1996) (quoting *In re Oetiker*, 977 F.2d 1443, 1445, 24 U.S.P.Q.2d 1443, 1444 (Fed. Cir. 1992)).

[3] *Id.* at 1583.

[4] Part IIB, 66 Fed. Reg. 1098 (2001).

-17-

Biology of the Cell (3rd ed. 1994) (copy enclosed, herein after Cell 3rd) and (4th ed. 2002) (copy enclosed, herein after Cell 4th). Figure 9-2 of Cell 3rd shows the steps at which eukaryotic gene expression can be controlled. The first step depicted is transcriptional control. Cell 3rd provides that "[f]or most genes transcriptional controls are paramount. This makes sense because, of all the possible control points illustrated in Figure 9-2, only transcriptional control ensures that no superfluous intermediates are synthesized." Cell 3rd at 403 (emphasis added). In addition, the text states that "Although controls on the initiation of gene transcription are the predominant form of regulation for most genes, other controls can act later in the pathway from RNA to protein to modulate the amount of gene product that is made." Cell 3rd at 453 (emphasis added). Thus, as established in Cell 3rd, the predominant mechanism for regulating the amount of protein produced is by regulating transcription initiation.

In Cell 4th, Figure 6-3 on page 302 illustrates the basic principle that there is a correlation between increased gene expression and increased protein expression. The accompanying text states that "a cell can change (or regulate) the expression of each of its genes according to the needs of the moment – *most obviously by controlling the production of its mRNA.*" Cell 4th at 302 (Emphasis added). Similarly, Figure 6-90 on page 364 of Cell 4th illustrates the path from gene to protein. The accompanying text states that while potentially each step can be regulated by the cell, "the initiation of transcription is the most common point for a cell to regulate the expression of each of its genes." Cell 4th at 364 (Emphasis added). This point is repeated on page 379, where the authors state that of all the possible points for regulating protein expression, "[f]or most genes transcriptional controls are paramount." Cell 4th at 379 (Emphasis added).

Further support for Applicants' position can be found in the textbook, Genes VI, (Benjamin Lewin, Genes VI (1997)) (copy enclosed) which states "having acknowledged that control of gene expression can occur at multiple stages, and that production of RNA cannot inevitably be equated with production of protein, it is clear that the overwhelming majority of regulatory events occur at the initiation of transcription." *Genes VI* at 847-848 (emphasis added).

Additional support is also found in Zhigang *et al.*, World Journal of Surgical Oncology 2:13, 2004 (copy enclosed). Zhigang studied the expression of prostate stem cell antigen (PSCA) protein and mRNA to validate it as a potential molecular target for diagnosis and treatment of human prostate cancer. The data showed "a high degree of correlation between PSCA protein

-18-

and mRNA expression" *Zhigang* at 4. Of the samples tested, 81 out of 87 showed a high degree of correlation between mRNA expression and protein expression. The authors conclude that "it is demonstrated that PSCA protein and mRNA overexpressed in human prostate cancer, and that the increased protein level of PSCA resulted from the upregulated transcription of its mRNA." *Zhigang* at 6. Even though the correlation between mRNA expression and protein expression occurred in 93% of the samples tested, not 100%, the authors state that "PSCA may be a promising molecular marker for the clinical prognosis of human Pca and a valuable target for diagnosis and therapy of this tumor." *Id.* at 7.

Further, Meric *et al.*, Molecular Cancer Therapeutics, vol. 1, 971-979 (2002) (copy enclosed) states the following:

> The **fundamental principle** of molecular therapeutics in cancer is to exploit the differences in <u>gene expression</u> between cancer cells and normal cells…[M]ost efforts have concentrated on identifying differences in gene expression at the level of mRNA, which can be attributable to either DNA amplification or to differences in transcription. Meric *et al.* at 971 (emphasis added).

Those of skill in the art would not be focusing on differences in gene expression between cancer cells and normal cells if there were no correlation between gene expression and protein expression.

Together, the declarations of Polakis, the accompanying references, and the excerpts and references provided above all establish that the accepted understanding in the art is that there is a reasonable correlation between changes in gene expression and the level of the encoded protein.

In addition to the supporting references previously submitted by Applicants, Applicants submit the following references to further support the assertion that changes in mRNA levels generally lead to corresponding changes in the level of the encoded polypeptide.

In a study by Wang *et al.* (Urol. Res. 2000; 28(5):308-15) (attached as Exhibit 3) the authors report that down-regulation of E-cadherin protein has been shown in various human tumors. *Id.* at Abstract. In the reported study, the authors examined the expression of cadherins and associated catenins at the mRNA level in paired tumor and non-neoplastic primary prostate cultures. They report that "[s]ix of seven cases of neoplastic cultures showed moderately-to-markedly decreased levels of E-cadherin and P-cadherin mRNA. Similar losses of alpha-catenin and beta-catenin mRNA were also observed." *Id.* As Applicants' assertion would predict, the

authors state that the mRNA measures showed "good correlation" with the results from protein measures. The authors conclude by stating that "this paper presents a coordinated down-regulation in the expression of E-cadherin and associated catenins at the mRNA and protein level in most of the cases studied." *Id.*

In a more recent study by Munaut *et al.* (Int. J. Cancer. 2003; 106(6):848-55) (attached as Exhibit 4) the authors report that vascular endothelial growth factor (VEGF) is expressed in 64-95% of glioblastomas (GBMs), and that VEGF receptors (VEGFR-1, its soluble form sVEGFR-1, VEGFR-2 and neuropilin-1) are expressed predominantly by endothelial cells. *Id.* at Abstract. The authors explain that infiltrating tumor cells and newly-formed capillaries progress through the extracellular matrix by local proteolysis involving matrix metalloproteinases (MMPs). In the present study, the authors "used quantitative RT-PCR, Western blot, gelatin zymography and immunohistochemistry to study the expression of VEGF, VEGFR-1, VEGFR-2, sVEGFR-1, neuropilin-1, MT1-MMP, MMP-2, MMP-9 and TIMP-2 in 20 human GBMs and 5 normal brains. The expression of these MMPs was markedly increased in most GBMs with excellent correlation between mRNA and protein levels." *Id.* Thus, the results support Applicants' assertion that changes in mRNA level lead to corresponding changes in protein level.

In another recent study, Hui *et al.* (Leuk. Lymphoma. 2003; 44(8):1385-94 (abstract attached as Exhibit 5) used real-time quantitative PCR and immunohistochemistry to evaluate cyclin D1 mRNA and protein expression levels in mantle cell lymphoma (MCL). *Id.* at Abstract. The authors report that seven of nine cases of possible MCL showed overexpression of cyclin D1 mRNA, while two cases showed no cyclin D1 mRNA increase. *Id.* Similarly, "[s]ix of the seven cyclin D1 mRNA overexpressing cases showed increased cyclin D1 protein on tissue array immunohistochemistry; one was technically suboptimal." *Id.* The authors conclude that the study "demonstrates good correlation and comparability between measure of cyclin D1 mRNA ... and cyclin D1 protein." *Id.* Thus, this reference supports Applicants' assertion.

In a recent study by Khal *et al.* (Int. J. Biochem. Cell Biol. 2005; 37(10):2196-206) (abstract attached as Exhibit 6) the authors report that atrophy of skeletal muscle is common in patients with cancer and results in increased morbidity and mortality. *Id.* at Abstract. To further understand the underlying mechanism, the authors studied the expression of the ubiquitin-proteasome pathway in cancer patient muscle using a competitive RT-PCR to measure

expression of mRNA for proteasome subunits C2 and C5, while protein expression was determined by western blotting. "Overall, both C2 and C5 gene expression was increased by about three-fold in skeletal muscle of cachectic cancer patients (average weight loss 14.5+/-2.5%), compared with that in patients without weight loss, with or without cancer. ... There was a good correlation between expression of proteasome 20Salpha subunits, detected by western blotting, and C2 and C5 mRNA, showing that increased gene expression resulted in increased protein synthesis." These findings support Applicants' assertion that changes in mRNA level lead to changes in protein level.

Maruyama *et al.* (Am. J. Patho. 1999; 155(3):815-22) (attached as Exhibit 7) investigated the expression of three Id proteins (Id-1, Id-2 and Id-3) in normal pancreas, in pancreatic cancer and in chronic pancreatitis (CP). The authors report that pancreatic cancer cell lines frequently coexpressed all three Ids, "exhibiting good correlation between Id mRNA and protein levels." *Id.* at Abstract. In addition, the authors teach that all three Id mRNA levels were expressed at high levels in pancreatic cancer samples compared to normal or CP samples. At the protein level, Id-1 and Id-2 staining was faint in normal tissue, while Id-3 ranged from weak to strong. In contrast, in the cancer tissues "many of the cancer cells exhibited abundant Id-1, Id-2, and Id-3 immunoreactivity," and Id-1 and Id-2 protein was increased significantly in the cancer cells by comparison to the respective controls, mirroring the overexpression at the mRNA level. Thus, the authors report that in both cell lines and tissue samples, increased mRNA levels leads to an increase in protein overexpression, supporting Applicants' assertion.

Support for Applicants' assertion is also found in an article by Caberlotto *et al.* (Neurosci. Lett. 1999; 256(3):191-4) (abstract attached as Exhibit 8). In a previous study, the authors investigated alterations of neuropeptide Y (NPY) mRNA expression in the Flinders Sensitive Line rats (FSL), an animal model of depression. *Id.* at Abstract. The authors reported that in the current study, that NPY-like immunoreactivity (NPY-LI) was decreased in the hippocampal CA region, and increased in the arcuate nucleus, and that fluoxetine treatment elevated NPY-LI in the arcuate and anterior cingulate cortex. The authors state that "[t]he results demonstrate a good correlation between NPY peptide and mRNA expression." Thus, increases and decreases in mRNA levels were reflected in corresponding changes in protein level.

-21-

Misrachi and Shemesh (Biol. Reprod. 1999; 61(3):776-84) (abstract attached as Exhibit 9) investigated their hypothesis that FSH regulates the bovine cervical prostaglandin E(2) (PGE(2)) synthesis that is known to be associated with cervical relaxation and opening at the time of estrus. *Id.* at Abstract. Cervical tissue from pre-estrous/estrous, luteal, and postovulatory cows were examined for the presence of bovine (b) FSH receptor (R) and its corresponding mRNA. The authors report that bFSHR mRNA in the cervix was maximal during pre-estrus/estrus, and that the level of FSHR protein was significantly higher in pre-estrous/estrous cervix than in other cervical tissues. *Id.* The authors state that "[t]here was a good correlation between the 75-kDa protein expression and its corresponding transcript of 2.55 kb throughout the estrous cycle as described by Northern blot analysis as well as RT-PCR." *Id.* Thus, changes in the level of mRNA for bFSHR led to corresponding changes in FSHR protein levels, a result which supports Applicants' assertion.

In a study by Stein *et al.* (J. Urol. 2000; 164(3 Pt 2):1026-30) (abstract attached as Exhibit 10), the authors studied the role of the regulation of calcium ion homeostasis in smooth muscle contractility. *Id.* at Abstract. The authors investigated the correlation between sarcoplasmic endoplasmic reticulum, calcium, magnesium, adenosine triphosphatase (SERCA) protein and gene expression, and the contractile properties in the same bladder. Partial bladder outlet obstructions were created in adult New Zealand white rabbits, which were divided into control, sham operated and obstructed groups. Stein *et al.* report that "[t]he relative intensities of signals for the Western [protein] and Northern [mRNA] blots demonstrated a strong correlation between protein and gene expression. ... The loss of SERCA protein expression is mediated by down-regulation in gene expression in the same bladder." *Id.* This report supports Applicants' assertion that changes in mRNA level, e.g. a decrease, lead to a corresponding change in the level of the encoded protein, *e.g.,* a decrease.

In an article by Gou and Xie (Zhonghua Jie He He Hu Xi Za Zhi. 2002; 25(6):337-40) (abstract attached as Exhibit 11) the authors investigated the expression of macrophage migration inhibitory factor (MIF) in human acute respiratory distress syndrome(ARDS) by examining the expression of MIF mRNA and protein in lung tissue in ARDS and normal persons. *Id.* at Abstract. The authors report "undetectable or weak MIF mRNA and protein expression in normal lungs. In contrast, there was marked upregulation of MIF mRNA and protein expression

-22-

in the ARDS lungs." *Id.* This is consistent with Applicants' assertion that a change in mRNA for a particular gene, *e.g.,* an increase, generally leads to a corresponding change in the level of protein expression, *e.g.,* an increase.

These studies are representative of <u>numerous</u> published studies which support Applicants' assertion that changes in mRNA level generally lead to corresponding changes in the level of the expressed protein. Applicants submit herewith an addition 70 references (abstracts attached as Exhibit 12) which support Applicants' assertion.

In addition to these supporting references, Applicants also submit herewith additional references which offer support of Applicants' asserted utility by showing that, in general, mRNA expression levels correlate with protein expression levels.

For example, in an article by Futcher *et al.* (Mol. Cell Biol. 1999; 19(11):7357-68) (attached as Exhibit 13) the authors conducted a study of mRNA and protein expression in yeast. Futcher *et al.* report "a good correlation between protein abundance, mRNA abundance, and codon bias." *Id.* at Abstract.

In a study which is more closely related to Applicants' asserted utility, Godbout *et al.* (J. Biol. Chem. 1998; 273(33)21161-8) (abstract attached as Exhibit 14) studied the DEAD box gene, DDX1, in retinoblastoma and neuroblastoma tumor cell lines. The authors report that "there is a good correlation with DDX1 gene copy number, DDX1 transcript levels, and DDX1 protein levels in all cell lines studied." *Id.* Thus, in these cancer cell lines, DDX1 mRNA and protein levels are correlated.

Similarly, in an article by Papotti *et al.* (Virchows Arch. 2002; 440(5):461-75) (abstract attached as Exhibit 15) the authors examined the expression of three somatostatin receptors (SSTR) at the mRNA and protein level in forty-six tumors. *Id.* at Abstract. The authors report a "good correlation between RT-PCR [mRNA level] and IHC [protein level] data on SSTR types 2, 3, and 5." *Id.*

Van der Wilt *et al.* (Eur. J. Cancer. 2003; 39(5):691-7) (abstract attached as Exhibit 16) studied deoxycytidine kinase (dCK) in seven cell lines, sixteen acute myeloid leukemia samples, ten human liver samples, and eleven human liver metastases of colorectal cancer origin. *Id.* at Abstract. The authors report that "enzyme activity and protein expression levels of dCK in cell

-23-

lines were closely related to the mRNA expression levels" and that there was a "good correlation between the different dCK measurements in malignant cells and tumors." *Id.*

Grenback *et al.* (Regul. Pept. 2004; 117(2):127-39) (abstract attached as Exhibit 17) studied the level of galanin in human pituitary adenomas using a specific radioimmunoassay. *Id.* at Abstract. The authors report that "[i]n the tumors analyzed with in situ hybridization there was a good correlation between galanin peptide levels and galanin mRNA expression." *Id.*

Similarly, Shen *et al.* (Blood. 2004; 104(9):2936-9) (abstract attached as Exhibit 18) examined the level of B-cell lymphoma 2 (BCL2) protein expression in germinal center (GC) B-cells and diffuse large B-cell lymphoma (DLBCL). *Id.* at Abstract. The authors report that "GC cells had low expression commensurate with the low protein expression level" and that in DLBCL the level of BCL2 mRNA and protein expression showed "in general, a good correlation." *Id.*

Likewise, in an article by Fu *et al.* (Blood 2005; 106(13):4315-21) (abstract attached as Exhibit 19) the authors report that six mantle cell lymphomas studied "expressed either cyclin D2 (2 cases) or cyclin D3 (4 cases)." *Id.* at Abstract. "There was a good correlation between cyclin D protein expression and the corresponding mRNA expression levels by gene expression analysis." *Id.*

These examples are only a few of the many references Applicants could cite in rebuttal to the PTO's arguments. Applicants submit herewith 26 additional references (abstracts attached as Exhibit 20) which also support Applicants' assertion in that they report a correlation between the level of mRNA and corresponding protein, contrary to the assertion of the PTO that mRNA and protein levels are not correlated.

Applicants note that the new references submitted in the Information Disclosure Statement focus on the correlation between mRNA expression and protein expression levels, and for the most part do not examine gene amplification. However, those few references that actually looked at gene amplification **did** find a correlation between gene amplification and increased mRNA and protein expression levels.

For example, Bea *et al.* (Cancer Res. 2001; 61(6):2409-12) (abstract attached in Exhibit 12) investigated gene amplification, mRNA expression, and protein expression of the putative oncogene BMI-1 in human lymphoma samples. The authors found BMI-1 gene amplification in

-24-

four mantle cell lymphomas (MCLs). Bea *et al.* report that "[t]he **four tumors with gene amplification showed significantly higher mRNA levels** than other MCLs and NHLs with the BMI-1 gene in germline configuration" (Abstract; emphasis added). Applicants note that the fact that five additional MCLs also showed very high mRNA levels without gene amplification does <u>not</u> disprove Applicants' position, because one of skill in the art would understand that there can be more than one cause of mRNA overexpression. The issue is not whether mRNA overexpression is always, or even typically caused by gene amplification, but rather, whether gene amplification typically leads to overexpression. Bea *et al.* further note that the four MCLS with gene amplification of *BMI-1* "showed significantly higher levels of mRNA **and protein expression** compared with other lymphomas with *BMI-1* in germline configuration" (page 2411, col. 1; emphasis added). Thus Bea *et al.* supports Applicants' assertion that gene amplification is correlated with both increased mRNA and protein expression.

Godbout *et al.* (J. Biol. Chem. 1998; 273(33)21161-8) (abstract attached as Exhibit 14) studied the DEAD box gene, DDX1, in retinoblastoma and neuroblastoma tumor cell lines. The authors report that "**there is a good correlation with DDX1 gene copy number, DDX1 transcript levels, and DDX1 protein levels in all cell lines studied**" (Abstract). Thus Godbout *et al.* also supports Applicants' assertion that gene amplification is correlated with both increased mRNA and protein expression.

Applicants note that while Fu *et al.* (Blood 2005; 106(13):4315-21) (abstract attached as Exhibit 19) found increased mRNA and protein expression of cyclin D2 and cyclin D3 in the absence of gene amplification, this result proves only that increased mRNA and protein expression levels can result from causes <u>other</u> than gene amplification. As Applicants do not assert that gene amplification is the <u>only</u> cause of increased mRNA and protein expression levels, this result does not disprove Applicants assertion that that increased gene amplification, in general, is correlated with increased mRNA and protein expression.

In summary, Applicants submit herewith a total of 118 references in addition to the declarations and references already of record which support Applicants' asserted utility, either directly or indirectly. These references, together with the previous Orntoft, Hyman, Pollack and Hanna references of record, support the assertion that that in general, amplification of a particular gene leads to a corresponding change in the level of expression of the mRNA and encoded

-25-

protein. These references further support the assertion that in general, a change in mRNA expression level for a particular gene leads to a corresponding change in the level of expression of the encoded protein. As Applicants have previously acknowledged, the correlation between changes in mRNA level and protein level is not exact, and there are exceptions (*see, e.g.,* abstracts attached as Exhibit 21). However, Applicants remind the PTO that the asserted utility does not have to be established to a statistical certainty, or beyond a reasonable doubt. *See M.P.E.P.* at §2107.02, part VII (2004). Therefore, the fact that there are exceptions to the correlation between changes in mRNA and changes in protein does not provide a proper basis for rejecting Applicants' asserted utility. Applicants submit that considering the evidence as a whole, with the <u>overwhelming majority</u> of the evidence supporting Applicants' asserted utility, a person of skill in the art would conclude that Applicants' asserted utility is "<u>more likely than not true</u>."

Applicants therefore respectfully request withdrawal of the rejections of Claims 28-35 and 38-40 under 35 U.S.C. §101 and 35 U.S.C. §112, first paragraph.
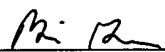
CONCLUSION

All claims pending in the present application are believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. **08-1641**, referencing Attorney's Docket No. **39780-2830 P1C38**). Please direct any calls in connection with this application to the undersigned at the number provided below.
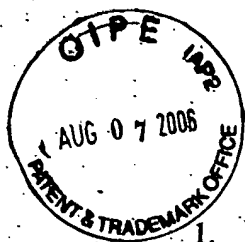
Respectfully submitted,

Date: August 7, 2006

By:_____
Barrie D. Greene (Reg. No. 46,740)

HELLER EHRMAN LLP
275 Middlefield Road
Menlo Park, California 94025-3506
Telephone: (650) 324-7000
Facsimile: (650) 324-0638

SV 2224180 v1

-27-

SECOND DECLARATION OF PAUL POLAKIS, Ph.D.

I, Paul Polakis, Ph.D., declare and say as follows:

1.	I am currently employed by Genentech, Inc. where my job title is Staff Scientist.

2.	Since joining Genentech in 1999, one of my primary responsibilities has been leading Genentech's Tumor Antigen Project, which is a large research project with a primary focus on identifying tumor cell markers that find use as targets for both the diagnosis and treatment of cancer in humans.

3.	As I stated in my previous Declaration dated May 7, 2004 (attached as Exhibit A), my laboratory has been employing a variety of techniques, including microarray analysis, to identify genes which are differentially expressed in human tumor tissue relative to normal human tissue. The primary purpose of this research is to identify proteins that are abundantly expressed on certain human tumor tissue(s) and that are either (i) not expressed, or (ii) expressed at detectably lower levels, on normal tissue(s).

4.	In the course of our research using microarray analysis, we have identified approximately 200 gene transcripts that are present in human tumor tissue at significantly higher levels than in normal human tissue. To date, we have successfully generated antibodies that bind to 31 of the tumor antigen proteins expressed from these differentially expressed gene transcripts and have used these antibodies to quantitatively determine the level of production of these tumor antigen proteins in both human tumor tissue and normal tissue. We have then quantitatively compared the levels of mRNA and protein in both the tumor and normal tissues analyzed. The results of these analyses are attached herewith as Exhibit B. In Exhibit B, "+" means that the mRNA or protein was detectably overexpressed in the tumor tissue relative to normal tissue and "-" means that no detectable overexpression was observed in the tumor tissue relative to normal tissue.

5.	As shown in Exhibit B, of the 31 genes identified as being detectably overexpressed in human tumor tissue as compared to normal human tissue at the mRNA level, 28 of them (i.e., greater than 90%) are also detectably overexpressed in human tumor tissue as compared to normal human tissue at the protein level. As such, in the cases where we have been able to quantitatively measure both (i) mRNA and (ii) protein levels in both (i) tumor tissue and (ii) normal tissue, we have observed that in the vast majority of cases, there is a very strong correlation between increases in mRNA expression and increases in the level of protein encoded by that mRNA.

6. Based upon my own experience accumulated in more than 20 years of research, including the data discussed in paragraphs 4-5 above and my knowledge of the relevant scientific literature, it is my considered scientific opinion that for human genes, an increased level of mRNA in a tumor tissue relative to a normal tissue more often than not correlates to a similar increase in abundance of the encoded protein in the tumor tissue relative to the normal tissue. In fact, it remains a generally accepted working assumption in molecular biology that increased mRNA levels are more often than not predictive of elevated levels of the encoded protein. In fact, an entire industry focusing on the research and development of therapeutic antibodies to treat a variety of human diseases, such as cancer, operates on this working assumption.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

Dated: _3-29-06_   By: _Paul Polakis_

Paul Polakis, Ph.D.

2

DECLARATION OF PAUL POLAKIS, Ph.D.

I, Paul Polakis, Ph.D., declare and say as follows:

1.   I was awarded a Ph.D. by the Department of Biochemistry of the Michigan State University in 1984. My scientific Curriculum Vitae is attached to and forms part of this Declaration (Exhibit A).

2.   I am currently employed by Genentech, Inc. where my job title is Staff Scientist. Since joining Genentech in 1999, one of my primary responsibilities has been leading Genentech's Tumor Antigen Project, which is a large research project with a primary focus on identifying tumor cell markers that find use as targets for both the diagnosis and treatment of cancer in humans.

3.   As part of the Tumor Antigen Project, my laboratory has been analyzing differential expression of various genes in tumor cells relative to normal cells. The purpose of this research is to identify proteins that are abundantly expressed on certain tumor cells and that are either (i) not expressed, or (ii) expressed at lower levels, on corresponding normal cells. We call such differentially expressed proteins "tumor antigen proteins". When such a tumor antigen protein is identified, one can produce an antibody that recognizes and binds to that protein. Such an antibody finds use in the diagnosis of human cancer and may ultimately serve as an effective therapeutic in the treatment of human cancer.

4.   In the course of the research conducted by Genentech's Tumor Antigen Project, we have employed a variety of scientific techniques for detecting and studying differential gene expression in human tumor cells relative to normal cells, at genomic DNA, mRNA and protein levels. An important example of one such technique is the well known and widely used technique of microarray analysis which has proven to be extremely useful for the identification of mRNA molecules that are differentially expressed in one tissue or cell type relative to another. In the course of our research using microarray analysis, we have identified approximately 200 gene transcripts that are present in human tumor cells at significantly higher levels than in corresponding normal human cells. To date, we have generated antibodies that bind to about 30 of the tumor antigen proteins expressed from these differentially expressed gene transcripts and have used these antibodies to quantitatively determine the level of production of these tumor antigen proteins in both human cancer cells and corresponding normal cells. We have then compared the levels of mRNA and protein in both the tumor and normal cells analyzed.

5.   From the mRNA and protein expression analyses described in paragraph 4 above, we have observed that there is a strong correlation between changes in the level of mRNA present in any particular cell type and the level of protein

expressed from that mRNA in that cell type. In approximately 80% of our observations we have found that increases in the level of a particular mRNA correlates with changes in the level of protein expressed from that mRNA when human tumor cells are compared with their corresponding normal cells.

6.   Based upon my own experience accumulated in more than 20 years of research, including the data discussed in paragraphs 4 and 5 above and my knowledge of the relevant scientific literature, it is my considered scientific opinion that for human genes, an increased level of mRNA in a tumor cell relative to a normal cell typically correlates to a similar increase in abundance of the encoded protein in the tumor cell relative to the normal cell. In fact, it remains a central dogma in molecular biology that increased mRNA levels are predictive of corresponding increased levels of the encoded protein. While there have been published reports of genes for which such a correlation does not exist, it is my opinion that such reports are exceptions to the commonly understood general rule that increased mRNA levels are predictive of corresponding increased levels of the encoded protein.

7.   I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

Dated: _5/07/04_   By: _Paul Polakis_

Paul Polakis, Ph.D.

SV 2031808 v1

2

CURRICULUM VITAE

PAUL G. POLAKIS
Staff Scientist
Genentech, Inc
1 DNA Way, MS#40
S. San Francisco, CA 94080

EDUCATION:

Ph.D., Biochemistry, Department of Biochemistry,
Michigan State University (1984)

B.S., Biology. College of Natural Science, Michigan State University (1977)

PROFESSIONAL EXPERIENCE:

2002-present	Staff Scientist, Genentech, Inc S. San Francisco, CA
1999- 2002	Senior Scientist, Genentech, Inc., S. San Francisco, CA
1997 -1999	Research Director Onyx Pharmaceuticals, Richmond, CA
1992- 1996	Senior Scientist, Project Leader, Onyx Pharmaceuticals, Richmond, CA
1991-1992	Senior Scientist, Chiron Corporation, Emeryville, CA.
1989-1991	Scientist, Cetus Corporation, Emeryville CA.
1987-1989	Postdoctoral Research Associate, Genentech, Inc., South SanFrancisco, CA.
1985-1987	Postdoctoral Research Associate, Department of Medicine, Duke University Medical Center, Durham, NC

1984-1985	Assistant Professor, Department of Chemistry, Oberlin College, Oberlin, Ohio
1980-1984	Graduate Research Assistant, Department of Biochemistry, Michigan State University East Lansing, Michigan

PUBLICATIONS:

1. Polakis, P. G. and Wilson, J. E. 1982 Purification of a Highly Bindable Rat Brain Hexokinase by High Performance Liquid Chromatography. Biochem. Biophys. Res. Commun. 107, 937-943.

2. Polakis, P.G. and Wilson, J. E. 1984 Proteolytic Dissection of Rat Brain Hexokinase: Determination of the Cleavage Pattern during Limited Digestion with Trypsin. Arch. Biochem. Biophys. 234, 341-352.

3. Polakis, P. G. and Wilson, J. E. 1985 An Intact Hydrophobic N-Terminal Sequence is Required for the Binding Rat Brain Hexokinase to Mitochondria. Arch. Biochem. Biophys. 236, 328-337.

4. Uhing, R.J., Polakis,P.G. and Snyderman, R. 1987 Isolaton of GTP-binding Proteins from Myeloid HL60 Cells. J. Biol. Chem. 262, 15575-15579.

5. Polakis, P.G., Uhing, R.J. and Snyderman, R. 1988 The Formylpeptide Chemoattractant Receptor Copurifies with a GTP-binding Protein Containing a Distinct 40 kDa Pertussis Toxin Substrate. J. Biol. Chem. 263, 4969-4979.

6. Uhing, R. J., Dillon, S., Polakis, P. G., Truett, A. P. and Snyderman, R. 1988 Chemoattractant Receptors and Signal Transduction Processes in Cellular and Molecular Aspects of Inflammation (Poste, G. and Crooke, S. T. eds.) pp 335-379.

7. Polakis, P.G., Evans, T. and Snyderman 1989 Multiple Chromatographic Forms of the Formylpeptide Chemoattractant Receptor and their Relationship to GTP-binding Proteins. Biochem. Biophys. Res. Commun. 161, 276-283.

8. Polakis, P. G., Snyderman, R. and Evans, T. 1989 Characterization of G25K, a GTP-binding Protein Containing a Novel Putative Nucleotide Binding Domain. Biochem. Biophys. Res. Comun. 160, 25-32.

9. Polakis, P., Weber,R.F., Nevins,B., Didsbury, J. Evans,T. and Snyderman, R. 1989 Identification of the ral and rac1 Gene Products, Low Molecular Mass GTP-binding Proteins from Human Platelets. J. Biol. Chem. 264, 16383-16389.

10. Snyderman, R., Perianin, A., Evans, T., Polakis, P. and Didsbury, J. 1989 G Proteins and Neutrophil Function. In ADP-Ribosylating Toxins and G Proteins: Insights into Signal Transduction. (J. Moss and M. Vaughn, eds.) Amer. Soc. Microbiol. pp. 295-323.

11. Hart, M.J., Polakis, P.G., Evans, T. and Cerrione, R.A. 1990 The Identification and Charaterization of an Epidermal Growth Factor-Stimulated Phosphorylation of a Specific Low Molecular Mass GTP-binding Protein in a Reconstituted Phospholipid Vesicle System. J. Biol. Chem. 265, 5990-6001.

12. Yatani, A., Okabe, K., Polakis, P. Halenbeck, R. McCormick, F. and Brown, A. M. 1990 ras p21 and GAP Inhibit Coupling of Muscarinic Receptors to Atrial K^+ Channels. Cell. 61, 769-776.

13. Munemitsu, S., Innis, M.A., Clark, R., McCormick, F., Ullrich, A. and Polakis, P.G. 1990 Molecular Cloning and Expression of a G25K cDNA, the Human Homolog of the Yeast Cell Cycle Gene CDC42. Mol. Cell. Biol. 10, 5977-5982.

14. Polakis, P.G. Rubinfeld, B. Evans, T. and McCormick, F. 1991 Purification of Plasma Membrane-Associated GTPase Activating Protein (GAP) Specific for rap-1/krev-1 from HL60 Cells. Proc. Natl. Acad. Sci. USA 88, 239-243.

15. Moran, M. F., Polakis, P., McCormick, F., Pawson, T. and Ellis, C. 1991 Protein Tyrosine Kinases Regulate the Phosphorylation, Protein Interactions, Subcellular Distribution, and Activity of p21ras GTPase Activating Protein. Mol. Cell. Biol. 11, 1804-1812

16. Rubinfeld, B., Wong, G., Bekesi, E. Wood, A. McCormick, F. and Polakis, P. G. 1991 A Synthetic Peptide Corresponding to a Sequence in the GTPase Activating Protein Inhibits p21[ras] Stimulation and Promotes Guanine Nucleotide Exchange. Internatl. J. Peptide and Prot. Res. 38, 47-53.

17. Rubinfeld, B., Munemitsu, S., Clark, R., Conroy, L., Watt, K., Crosier, W., McCormick, F., and Polakis, P. 1991 Molecular Cloning of a GTPase Activating Protein Specific for the Krev-1 Protein p21[rap1]. Cell 65, 1033-1042.

18. Zhang, K. Papageorge, A., G., Martin, P., Vass, W. C., Olah, Z., Polakis, P., McCormick, F. and Lowy, D, R. 1991 Heterogenous Amino Acids in RAS and Rap1A Specifying Sensitivity to GAP Proteins. Science 254, 1630-1634.

19. Martin, G., Yatani, A., Clark, R., Polakis, P., Brown, A. M. and McCormick, F. 1992 GAP Domains Responsible for p21[ras]-dependent Inhibition of Muscarinic Atrial K^+ Channel Currents. Science 255, 192-194.

20. McCormick, F., Martin, G. A., Clark, R., Bollag, G. and Polakis, P. 1992 Regulation of p21ras by GTPase Activating Proteins. Cold Spring Harbor Symposia on Quantitative Biology. Vol. 56, 237-241.

21. Pronk, G. B., Polakis, P., Wong, G., deVries-Smits, A. M., Bos J. L. and McCormick, F. 1992 p60[v-src] Can Associate with and Phosphorylate the p21[ras] GTPase Activating Protein. Oncogene 7, 389-394.

22. Polakis P. and McCormick, F. 1992 Interactions Between p21[ras] Proteins and Their GTPase Activating Proteins. In Cancer Surveys (Franks, L. M., ed.) 12, 25-42.

23. Wong, G., Muller, O., Clark, R., Conroy, L., Moran, M., Polakis,P. and McCormick, F. 1992 Molecular coloning and nucleic acid binding properties of the GAP-associated tyrosine phosphoprotein p62. Cell 69, 551-558.

24. Polakis, P., Rubinfeld, B. and McCormick, F. 1992 Phosphorylation of rap1GAP in vivo and by cAMP-dependent Kinase and the Cell Cycle p34^{cdc2} Kinase in vitro. J. Biol. Chem. 267, 10780-10785.

25. McCabe, P.C., Haubrauck, H., Polakis, P., McCormick, F., and Innis, M. A. 1992 Functional Interactions Between p21^{rap1A} and Components of the Budding pathway of Saccharomyces cerevisiae. Mol. Cell. Biol. 12, 4084-4092.

26. Rubinfeld, B., Crosier, W.J., Albert, I., Conroy,L., Clark, R., McCormick, F. and Polakis, P. 1992 Localization of the rap1GAP Catalytic Domain and Sites of Phosphorylation by Mutational Analysis. Mol. Cell. Biol. 12, 4634-4642.

27. Ando, S., Kaibuchi, K., Sasaki, K., Hiraoka, T., Nishiyama, T., Mizuno, T., Asada, M., Nunoi, H., Matsuda, I., Matsuura, Y., Polakis, P., McCormick, F. and Takai, Y. 1992 Post-translational processing of rac p21s is important both for their interaction with the GDP/GTP exchange proteins and for their activation of NADPH oxidase. J. Biol. Chem. 267, 25709-25713.

28. Janoueix-Lerosey, I., Polakis, P., Tavitian, A. and deGunzberg, J. 1992 Regulation of the GTPase activity of the ras-related rap2 protein. Biochem. Biophys. Res. Commun. 189, 455-464.

29. Polakis, P. 1993 GAPs Specific for the rap1/Krev-1 Protein. in GTP-binding Proteins: the ras-superfamily. (J.C. LaCale and F. McCormick, eds.) 445-452.

30. Polakis, P. and McCormick, F. 1993 Structural requirements for the interaction of p21^{ras} with GAP, exchange factors, and its bological effector target. J. Biol Chem. 268, 9157-9160.

31. Rubinfeld, B., Souza, B. Albert, I., Muller, O., Chamberlain, S., Masiarz, F., Munemitsu, S. and Polakis, P. 1993 Association of the APC gene product with beta- catenin. Science 262, 1731-1734.

32. Weiss, J., Rubinfeld, B., Polakis, P., McCormick, F. Cavenee, W. A. and Arden, K. 1993 The gene for human rap1-GTPase activating protein (rap1GAP) maps to chromosome 1p35-1p36.1. Cytogenet. Cell Genet. 66, 18-21.

33. Sato, K. Y., Polakis, P., Haubruck, H., Fasching, C. L., McCormick, F. and Stanbridge, E. J. 1994 Analysis of the tumor suppressor acitvity of the K-rev gene in human tumor cell lines. Cancer Res. 54, 552-559.

34. Janoueix-Lerosey, I., Fontenay, M., Tobelem, G., Tavitian, A., Polakis, P. and DeGunzburg, J. 1994 Phosphorylation of rap1GAP during the cell cycle. Biochem. Biophys. Res. Commun. 202, 967-975

35. Munemitsu, S., Souza, B., Mueller, O., Albert, I., Rubinfeld, B., and Polakis, P. 1994 The APC gene product associates with microtubules in vivo and affects their assembly in vitro. Cancer Res. 54, 3676-3681.

36. Rubinfeld, B. and Polakis, P. 1995 Purification of baculovirus produced rap1GAP. Methods Enz. 255,31

37. Polakis, P. 1995 Mutations in the APC gene and their implications for protein structure and function. Current Opinions in Genetics and Development 5, 66-71

38. Rubinfeld, B., Souza, B., Albert, I., Munemitsu, S. and Polakis P. 1995 The APC protein and E-cadherin form similar but independent complexes with α-catenin, β-catenin and Plakoglobin. J. Biol. Chem. 270, 5549-5555

39. Munemitsu, S., Albert, I., Souza, B., Rubinfeld, B., and Polakis, P. 1995 Regulation of intracellular β-catenin levels by the APC tumor suppressor gene. Proc. Natl. Acad. Sci. 92, 3046-3050.

40. Lock, P., Fumagalli, S., Polakis, P. McCormick, F. and Courtneidge, S. A. 1996 The human p62 cDNA encodes Sam68 and not the rasGAP-associated p62 protein. Cell 84, 23-24.

41. Papkoff, J., Rubinfeld, B., Schryver, B. and Polakis, P. 1996 Wnt-1 regulates free pools of catenins and stabilizes APC-catenin complexes. Mol. Cell. Biol. 16, 2128-2134.

42. Rubinfeld, B., Albert, I., Porfiri, E., Fiol, C., Munemitsu, S. and Polakis, P. 1996 Binding of GSK3β to the APC-β-catenin complex and regulation of complex assembly. Science 272, 1023-1026.

43. Munemitsu, S., Albert, I., Rubinfeld, B. and Polakis, P. 1996 Deletion of amino-terminal structure stabilizes β-catenin in vivo and promotes the hyperphosphorylation of the APC tumor suppressor protein. Mol. Cell. Biol.16, 4088-4094.

44. Hart, M. J., Callow, M. G., Sousa, B. and Polakis P. 1996 IQGAP1, a calmodulin binding protein witha rasGAP related domain, is a potential effector for cdc42Hs. EMBO J. 15, 2997-3005.

45. Nathke, I. S., Adams, C. L., Polakis, P., Sellin, J. and Nelson, W. J. 1996 The adenomatous polyposis coli (APC) tumor suppressor protein is localized to plasma membrane sites involved in active epithelial cell migration. J. Cell. Biol. 134, 165-180.

46. Hart, M. J., Sharma, S., elMasry, N., Qui, R-G., McCabe, P., Polakis, P. and Bollag, G. 1996 Identification of a novel guanine nucleotide exchange factor for the rho GTPase. J. Biol. Chem. 271, 25452.

47. Thomas JE, Smith M, Rubinfeld B, Gutowski M, Beckmann RP, and Polakis P. 1996 Subcellular localization and analysis of apparent 180-kDa and 220-kDa proteins of the breast cancer susceptibility gene, BRCA1. J. Biol. Chem. 1996 271, 28630-28635

48. Hayashi, S., Rubinfeld, B., Souza, B., Polakis, P., Wieschaus, E., and Levine, A. 1997 A Drosophila homolog of the tumor suppressor adenomatous polyposis coli

down-regulates β-catenin but its zygotic expression is not essential for the regulation of armadillo. Proc. Natl. Acad. Sci. 94, 242-247.

49. Vleminckx, K., Rubinfeld, B., **Polakis, P.** and Gumbiner, B. 1997 The APC tumor suppressor protein induces a new axis in Xenopus embryos. J. Cell. Biol. 136, 411-420.

50. Rubinfeld, B., Robbins, P., El-Gamil, M., Albert, I., Porfiri, P. and **Polakis, P.** 1997 Stabilization of β-catenin by genetic defects in melanoma cell lines. Science 275, 1790-1792.

51. **Polakis, P.** The adenomatous polyposis coli (APC) tumor suppressor. 1997 Biochem. Biophys. Acta, 1332, F127-F147.

52. Rubinfeld, B., Albert, I., Porfiri, E., Munemitsu, S., and **Polakis, P** 1997 Loss of β-catenin regulation by the APC tumor suppressor protein correlates with loss of structure due to common somatic mutations of the gene. Cancer Res. 57, 4624-4630.

53. Porfiri, E., Rubinfeld, B., Albert, I., Hovanes. K., Waterman, M., and **Polakis, P.** 1997 Induction of a β-catenin-LEF-1 complex by wnt-1 and transforming mutants of β-catenin. Oncogene 15, 2833-2839.

54. Thomas JE, Smith M, Tonkinson JL, Rubinfeld B, and **Polakis P.,** 1997 Induction of phosphorylation on BRCA1 during the cell cycle and after DNA damage. Cell Growth Differ. 8, 801-809.

55. Hart, M., de los Santos, R., Albert, I., Rubinfeld, B., and **Polakis P.,** 1998 Down regulation of β-catenin by human Axin and its association with the adenomatous polyposis coli (APC) tumor suppressor, β-catenin and glycogen synthase kinase 3β. Current Biology 8, 573-581.

56. **Polakis, P.** 1998 The oncogenic activation of β-catenin. Current Opinions in Genetics and Development 9, 15-21

57. Matt Hart, Jean-Paul Concordet, Irina Lassot, Iris Albert, Rico del los Santos, Herve Durand, Christine Perret, Bonnee Rubinfled, Florence Margottin, Richard Benarous and **Paul Polakis.** 1999 The F-box protein β–TrCP associates with phosphorylated β–catenin and regulates its activity in the cell. Current Biology 9, 207-10.

58. Howard C. Crawford, Barbara M. Fingleton, Bonnee Rubinfeld, **Paul Polakis** and Lynn M. Matrisian 1999 The metalloproteinase matrilysin is a target of β–catenin transactivation in intestinal tumours. Oncogene 18, 2883-91.

59. Meng J, Glick JL, **Polakis P,** Casey PJ. 1999 Functional interaction between Galpha(z) and Rap1GAP suggests a novel form of cellular cross-talk. J Biol Chem. 17, 36663-9

60. Vijayasurian Easwaran, Virginia Song, Paul Polakis and Steve Byers 1999 The ubiquitin-proteosome pathway and serine kinase activity modulate APC mediated regulation of β-catenin-LEF signaling. **J. Biol. Chem. 274(23):16641-5.**

61 Polakis P, Hart M and Rubinfeld B. 1999 Defects in the regulation of beta-catenin
in colorectal cancer. **Adv Exp Med Biol. 470, 23-32**

62 Shen Z, Batzer A, Koehler JA, Polakis P, Schlessinger J, Lydon NB, Moran MF. 1999 Evidence for SH3 domain directed binding and phosphorylation of Sam68 by Src. **Oncogene. 18, 4647-53**

64. Thomas GM, Frame S, Goedert M, Nathke I, Polakis P, Cohen P. 1999 A GSK3- binding peptide from FRAT1 selectively inhibits the GSK3-catalysed phosphorylation of axin and beta-catenin. **FEBS Lett. 458, 247-51.**

65. Peifer M, Polakis P. 2000 Wnt signaling in oncogenesis and embryogenesis--a look outside the nucleus. **Science 287,1606-9.**

66. Polakis P. 2000 Wnt signaling and cancer. **Genes Dev;14, 1837-1851.**

67. Spink KE, Polakis P, Weis WI 2000 Structural basis of the Axin-adenomatous polyposis coli interaction. **EMBO J 19, 2270-2279.**

68. Szeto, W., Jiang, W., Tice, D.A., Rubinfeld, B., Hollingshead, P.G., Fong, S.E., Dugger, D.L., Pham, T., Yansura, D.E., Wong, T.A., Grimaldi, J.C., Corpuz, R.T., Singh J.S., Frantz, G.D., Devaux, B., Crowley, C.W., Schwall, R.H., Eberhard, D.A.,
Rastelli, L., Polakis, P. and Pennica, D. 2001 Overexpression of the Retinoic Acid-
Responsive Gene Stra6 in Human Cancers and its Synergistic Induction by Wnt-1 and
Retinoic Acid. **Cancer Res 61, 4197-4204.**

69. Rubinfeld B, Tice DA, Polakis P. 2001 Axin dependent phosphorylation of the adenomatous polyposis coli protein mediated by casein kinase 1 epsilon. **J Biol Chem
276, 39037-39045.**

70. Polakis P. 2001 More than one way to skin a catenin. **Cell 2001 105, 563-566.**

71. Tice DA, Soloviev I, Polakis P. 2002 Activation of the Wnt Pathway Interferes withSerum Response Element-driven Transcription of Immediate Early Genes. **J Biol.
Chem. 277, 6118-6123.**

72. Tice DA, Szeto W, Soloviev I, Rubinfeld B, Fong SE, Dugger DL, Winer J,

Williams PM, Wieand D, Smith V, Schwall RH, Pennnica D, Polakis P. 2002 Synergistic activation of tumor antigens by wnt-1 signaling and retinoic acid revealed by gene expression profiling. J Biol Chem. 277, 14329-14335.

73. Polakis, P. 2002 Casein kinase I: A wnt'er of disconnect. Curr. Biol. 12, R499.

74. Mao, W. , Luis, E., Ross, S., Silva, J., Tan, C., Crowley, C., Chui, C., Franz, G., Senter, P., Koeppen, H., Polakis, P. 2004 EphB2 as a therapeutic antibody drug target for the treatment of colorectal cancer. Cancer Res. 64, 781-788.

75. Shibamoto, S., Winer, J., Williams, M., Polakis, P. 2003 A Blockade in Wnt signaling is activated following the differentiation of F9 teratocarcinoma cells. Exp. Cell Res. 2921 1-20.

76. Zhang Y, Eberhard DA, Frantz GD, Dowd P, Wu TD, Zhou Y, Watanabe C, Luoh SM, Polakis P, Hillan KJ, Wood WI, Zhang Z. 2004 GEPIS--quantitative gene expression profiling in normal and cancer tissues. Bioinformatics, April 8

	tumor mRNA	tumor IHC
UNQ2525	+	+
UNQ2378	+	+
UNQ972	+	-
UNQ97671	+	+
UNQ2964	+	+
UNQ323	+	+
UNQ1655	+	+
UNQ2333	+	+
UNQ9638	+	+
UNQ8209	+	+
UNQ6507	+	+
UNQ8196	+	+
UNQ9109	+	+
UNQ100	+	+
UNQ178	+	+
UNQ1477	+	+
UNQ1839	+	+
UNQ2079	+	+
UNQ8782	+	+
UNQ9646	+	-
UNQ111	+	+
UNQ3079	+	+
UNQ8175	+	+
UNQ9509	+	+
UNQ10978	+	-
UNQ2103	+	+
UNQ1563	+	+
UNQ16188	+	+
UNQ13589	+	+
UNQ1078	+	+
UNQ879	+	+